(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 662 946 B2

(12) **NEW EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the opposition decision:
12.12.2001 Bulletin 2001/50

(45) Mention of the grant of the patent:
11.03.1998 Bulletin 1998/11

(21) Application number: **93921002.7**

(22) Date of filing: **27.09.1993**

(51) Int Cl.[7]: **C07C 57/13**, A61K 7/48, A61K 31/19, A61K 31/20

(86) International application number:
PCT/GB93/02006

(87) International publication number:
WO 94/07837 (14.04.1994 Gazette 1994/09)

(54) UNSATURATED ALIPHATIC DICARBOXYLIC ACIDS

UNGESÄTTIGTE ALIPHATISCHE DICARBONSÄUREN

ACIDES DICARBOXYLIQUES ALIPHATIQUES INSATURES

(84) Designated Contracting States:
AT BE CH DE DK ES FR GB GR IE IT LI NL PT SE

(30) Priority: **30.09.1992 GB 9220667**

(43) Date of publication of application:
19.07.1995 Bulletin 1995/29

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Description

[0001] This invention relates to unsaturated dioic acids, that is, unsaturated aliphatic dicarboxylic acids, especially C_8-C_{22} compounds, particularly, but not exclusively, C_{16} and C_{18} unsaturated dioic acids (i.e. those having 16 or 18 carbon atoms), and concerns a method of producing dioic acids, and their use in the treatment of skin for medical and cosmetic purposes, such as acne treatment and skin lightening.

Background of the invention

[0002] It is known to use saturated dioic acids (having the general formula COOH $(CH_2)_nCOOH$) for the treatment of skin for medical and cosmetic purposes. For example, US 4386104 (Nazzaro-Porro) discloses saturated dioic acids, containing 7 to 13 carbon atoms, for the treament of acne and other skin conditions,

[0003] In pacticular, the C_9 saturated dioic acid ("azelaic" acid) having the formula $COOH(CH_2)_7COOH$, is frequently cited as being effective in the treatment of acne and other skin conditions, and in the lightening of skin.

[0004] Thus far, unsaturated dioic acids have not been readily commercially available, although a method of synthesising certain unsaturated dioic acids is disclosed in EP 0229252. Indeed some of this class of molecules have not previously been described. However, it is known that certain unsaturated dioic acids, particularly certain C_6, C_8, C_{10} and C_{12} mono-unsaturated dioic acids can be detected in the urine of patients with medium chain acyl-CoA dehydrogenase deficiency (Jin & Tserng [1989] Journal of Lipid Research 30, 1612-1619 and Tserng et al., [1990] Journal of Lipid Research 31, 763-771). Certain other unsaturated dioic acids are disclosed in various other publications.

[0005] EP 0341796 discloses a microbial route using Candida cloacae beta-oxidation mutants for the production of saturated dioic acids from longer chain saturated fatty acids (monocarboxylic acids) or triglycerides. The present inventors have now produced, using the mutants disclosed in EP0341796, certain unsaturated dioic acids, some of which are novel per se, which have been found to have surprisingly enhanced properties for use in the treatment of skin for medical and cosmetic purposes.

Summary of the invention

[0006] Surprisingly, it has been found by the present inventors that unsaturated dioic acids in general (especially C_{16} and C_{18} unsaturated dioic acids) possess much greater activity than their saturated dioic acid counterparts, as anti-microbial agents and as cosmetic agents.

[0007] The prior art teaches that conditions susceptible to treatment with dioic acids include: acne (US 4386104); wrinkles (EP 336880); malignant melanoma (US 4818768); dermatoses (EP 229654); hyperpigmentary dermatosis and eczema (US 4292326); rosacea (EP 890308); lentigo (JP 91024412) and seborrhoea (DE 3133425) and impetigo.

[0008] Similarily, the prior art teaches that some dioic acid derivatives are also effective in the treatment of certain conditions. Such compounds include esters and salts (US 4818768) and mercapto-derivatives of dioic acids (US 4292326). Other references to the utility of dioic acid derivatives may be found, for example, in JP 58170713, EP 0297436 and EP 0305407. German patent application No. DE 40 33 567 discloses, inter alia, mono ester derivatives of C_3-C_{14} (main hydrocarbon chain) dioic acids (which may be saturated or unsaturated) as sebosuppressive agents for use in cosmetic or pharmaceutical applications for topical use on the hair and skin.

[0009] Thus in a first aspect the invention provides a pharmaceutical or cosmetic composition as defined in claim 1.

[0010] The term "main hydrocarbon chain", used with respect to dioic acid derivatives, is intended to refer to that part of the molecule situated between the oxygen atoms of the two carboxylic acid groups (or the derivatised remnants thereof). Thus, for example, derivatives having the formulae R-OOC-CH_2-COO-R^1 and R-OOC-CH_2-CH_2-COO-R^1 would be described as having C_3 and C_4 main hydrocarbon chains respectively.

[0011] The derivatives may be, for example, alcohols, substituted or unsubstituted amides, mono- or diesters (aryl or alkyl. especially lower alkyl esters).

[0012] The unsaturated dioic acids employed in this method aspect of the invention contain 8 to 22 carbon atoms, most preferably 16 or 18 carbon atoms. The unsaturated dioic acid derivative preferably contains 16 or 18 carbon atoms in the main hydrocarbon chain.

[0013] Certain unsaturated dioic acids are found to be particularly active against Propionibacterium acnes (P. acnes) and Staphylococcus aureus (Scaph. aureus). Thus. in general, the method of the invention may also be found useful in treating any condition where P. acnes an/or Staph. aureus is known, or suspected, to be involved in causation. maintenance or exacerbation of that condition.

[0014] In accordance with the invention. unsaturated dioic acids can be used in the treatment of a wide range of skin conditions, such as acne etc, as discussed above in connection with the prior art. Similarily, references to derivatives are intended to refer to derivatives such as esters and salts, as discussed in connection with the prior art.

[0015] Other conditions which may be susceptible to improvement by the use of unsaturated dioic acids or their

derivatives include dandruff and the presence of body odour.

[0016] In addition it has been shown by the present inventors that unsaturated dioic acids are surprisingly effective as inhibitors of tyrosinase, and as inhibitors of melanin synthesis by cultivated melanoma cells, in tests used to screen compounds for activity as skin-lightening agents.

[0017] Therefore the invention provides in a second aspect, a method of lightening skin, as defined in claim 13.

[0018] In a third aspect, the invention provides a method of treating human skin for cosmetic purposes, as defined in claim 10.

[0019] Typically compositions in accordance with the invention will be formulated for topical application to the skin. The unsaturated dioic acids and their derivatives are readily incorporated into such compositions which, for example, may take the form of creams, lotions or gels. Suitable formulations are very well known to those skilled in the art and are disclosed, for example, in US 4818768.

[0020] Another aspect of the invention thus provides a method of making a therapeutic or cosmetic composition for topical application to the skin, as defined in claim 20.

[0021] The invention further provides use of an unsaturated aliphatic dioic acid or a derivative of an unsaturated aliphatic dioic acid, as defined in each of claims 14, 17 and 18.

[0022] The invention also provides an unsaturated aliphatic dioic acid, or a derivative thereof, as defined in claim 15.

[0023] The dioic acids used in such compositions may be prepared by the novel method disclosed below. Alternatively they may be prepared using known methods, for example as disclosed in EP 0 296 506, or as taught by Uemura et al. (1988, Proceedings of World Conference on Biotechnology for the Fats and Oil Industry, American Oil Chemists Society). Buhler & Schlinder (1984, in "Aliphatic Hydrocarbons in Biotechnology, Rehm & Reed (Eds.) 169, Verlag Chemie, Weinheim) or by Picataggio et al. (1992, Biotechnology 10, 894-898).

[0024] Certain unsaturated dioic acids may conveniently be produced from longer chain unsaturated substrates using the mutants dislosed in EP 0341796, which previously have been used to make saturated compounds. Therefore, in a further aspect, the invention provides a method of preparing unsaturated dioic acids, as defined in claim 22.

[0025] Preferably the unsaturated dioic acids produced are C_8-C_{22}, most preferably C_{16} or C_{18}.

[0026] Yeasts suitable for the purpose are disclosed in EP 0341796 and in Casey et al., ((1990) Journal of General Microbiology 136, 1197-1202). Such strains (Candida cloacae 5GLA12, abbreviated to "LA12") exhibit limited or reduced beta-oxidation activity.

[0027] Conveniently the yeasts are supplied with unsaturated fatty acids in the form of esters, preferably as triglyceride esters such as oil. Particularly suitable examples include unsaturated oils such as sunflower oil and olive oil.

[0028] Preferably, the oils used as starting materials are triglycerides in which the predominant unsaturated long chain fatty acid is a C_{16-22}, or more preferably, a C_{20} or C_{18} compound. Preferably the substrate material is predominantly poly-unsaturated. Fermentation by yeast strains LA12 can result in the production of mixtures of chain-shortened. unsaturated dioic acids (typically C_8-C_{18} compounds). These mixed products can be separated into fractions. for example by differential solvent extraction.

[0029] If one assumes that there is random removal of C_2 units during beta-oxidation, and that no isomerisation of the products occurs. the following products may be predicted to be formed when using oleic acid as a substrate:

cis-7-hexadecene dioic acid; cis-5-tetradecene dioic acid: cis-7-tetradecene dioic acid. cis-3-dodecene dioic acid: cis-5-dodecene dioic acid; cis-3-decene dioic acid: cis-5-decene dioic acid and cis-3-octene dioic acid.

[0030] From linoleic acid, the following products may be expected:

cis-6. 9-hexadecadiene dioic acid: cis-4, 7-hexadecaciene dioic acid: cis-5,8-tetradecadiene dioic acid. cis-4, 7-tetradecadiene dioic acid: cis-2, 5-tetradecadiene dioic acid; cis-3. 6-dodecadiene dioic acid: cis-4. 7-dodecadiene dioic acid: cis-2, 5-dodecadiene dioic acid: cis-3, 6-decadiene dioic acid; cis-2. 5-decadiene dioic acid: cis-2. 5-octadiene dioic acid: cis-4-decene dioic acid and cis-2-octene dioic acid.

[0031] Likewise, the predicted products using linolenic acid as a starting material are as follows:

cis-4 7, 10-hexadecatriene dioic acid; cis-6, 9, 12-hexadecatriene dioic acid: cis-2, 5, 8-tetradecatriene dioic acid: cis-4. 7, 10-tetradecatriene dioic acid: cis-2, 5, 8-dodetatriene dioic acid: cis-3, 6-dodecadiene dioic acid: cis-2, 5. 8-decatriene dioic acid, cis-3, 6-decadiene dioic acid: cis-4-decene dioic acid cis-2,5-octadiene dioic acid: cis-4-octene dioic acid and cis-2-octene dioic acid

[0032] In all cases, the product mixture will contain small amounts of products of the same chain length as the starting compound.

[0033] Indeed, whilst the preferred substrates are fatty acid esters (particularly C_{18} fatty acid esters), the products of the fermentation depend upon the starting substrate. Thus, by varying the substrate, a whole range of unsaturated dioic acids may be prepared.

[0034] Some suitable substrates are identified in EP 0 229 252 and include C_{10}-C_{24} alkenes and other unsaturated hydrocarbons such as unsaturated alkanols (especially C_{16}, C_{18} and C_{22} unsaturated alkanols), the corresponding mono-carboxylic acids, or their hydroxycarboxylic acid derivatives.

[0035] Naturally, where trans-unsaturated compounds are the starting compounds, trans-unsaturated products will

result.

[0036] It is a highly preferred feature that the yeast employed for tne process is not propagated under conditions of nitrogen limitation. Instead, (unlike the method described in EP 0341796), the yeast is grown under conditions which are comparatively enriched for nitrogen, wherein alteration of pH affects the chain shortening beta oxidation activity of the organism.

[0037] Thus, it is a found tha the product profile of the fermentation process may conveniently be modified by alteration of the pH of the fermentation medium during the production of the unsaturated dioic acids. In particular, it is possible to alter the relative concentrations of the different lengths of dioic acid molecules in this way. For example, by reducing the pH from 7.5 to 7.1 during fermentation of olive oil, it is possible to increase the relative amount of the C_{12} unsaturated dioic acid.

[0038] This is significant because certain fractions of the fermentation products may have especially advantageous properties for particular intended uses.

[0039] The different fractions of different products may be obtained from the culture medium by extracting with diethyl ether after adjustment of the aqueous phase to various different acidic pHs.

[0040] The different aspects of the invention can be better understood by reference to the following illustrative examples and drawings in which:

Figure 1 is a graph of dioic acid concentration (grams per litre) against time, using sunflower oil as a substrate;

Figure 2 is a graph of dioic acid concentration (grams per litre) against time, using olive oil as a substrate;

Figure 3 is a graph of dioic acid concentration (grams per litre) against time, using olive oil as a substrate with altered pH conditions; and

Figure 4 is a bar chart showing percentage melanin reduction for medium chain dioic acids obtained from sunflower oil.

Example I - Production of Medium chain unsaturated dioic acids by fermentation

[0041] A beta-oxidation mutant of Candida cloacae produced by mutagensis using nitrosoguanidine (mutant LA12, see EP0341796 and see also Casey et al., J. Gen. Microbiol (1990), 136, 1197-1202) was used to produce C_8-C_{14} unsaturated dioic acids from triglycerides such as olive oil and sunflower oil which contain high levels of unsaturated fatty acids.

[0042] A chemically defined medium was used as shown below:-

Sucrose	20g/l	)
$(NH_4)_2HPO_4$	6g/l	)
KH_2PO_4	6.4g/l	)
Na_2SO_4	1.5g/l	) autoclave 20
Triglyceride	10-40ml/l	) mins at 121°C
(eg olive oil or sunflower oil)		

$ZnSO_4.7H_2O$	20mg/l	)	
$MnSO_4 4H_2O$	20mg/l	)	
$FeSO_4.7H_2O$	20mg/l	)	
$MgCl_2.6H_2O$	2/gl	)	filter sterilise
Biotin	100mg/l	)	and add aseptically
Pantothenate	6mg/l	)	when fermenter cool
Thiamine	8mg/l	)	
Nicotinic acid	30mg/l	)	
Pyridoxine	20mg/l	)	

The fermenter conditions were:

Growth pH:	6.8	)	maintained by
Production pH:	7.4-7.5	)	auto-addition of
Temperature:	30°C	)	10N NaOH
Aeration:	0.1 v/v/m air		
Impeller speed:	800-1000 rpm		
Fermenter volume:	2.5L		
Inoculum:	2%		
Fermenter type:	LSL fitted with foam breaker		

[0043] The medium (2.5L) was inoculated with 2% (v/v) of a 24 hr culture of Candida cloacae beta-oxidation mutant LA12 grown on yeast extract (5g/l), sucrose(10g/l), peptone (59/l) medium. The culture was grown for 20 hrs at pH 6.8 then 20ml/l of oil was added and the pH increased to 7.4-7.6 to initiate production of the medium chain unsaturated dioic acids. The oil was either sunflower oil or silica-purified olive oil. During production of the dioic acids, the RQ (respiratory quotient) value fell to about 0.6. Aliquots (10-20ml) of fermenter broth were removed dally for lipid analysis and additional oil was added as required.

[0044] The fermentation was harvested when production ceased at 8-12 days.

[0045] Medium chain unsaturated dioic acids were isolated from fermenter broths by acidification to pH 6 with HCl then extraction with diethyl ether to isolate a C_{12}-C_{14} rich fraction. The broth was then further acidified with HCl to ca. pH2.0 and further extracted with diethyl ether to isolate a C_8-C_{10} rich fraction. For isolation of the mixed acids the broth pH was decreased from 7.5 to ca. 2.0 in one step then extracted with diethyl ether.

[0046] Solvent was removed from the dioic acid fractions by rotary evaporation.

[0047] A time course of medium chain unsaturated dioic acid production from sunflower oil (SFO) and silica-purified olive oil (OO) is shown in Figures 1 and 2 respectively.

[0048] Figure 1 shows the production of C_8-C_{14} unsaturated dioic acids individually and in total, using sunflower of as the substrate. The rate of production of unsaturated medium chain dioic acids increased rapidly between days 3 and 4 but declined virtually to zero by day 8, such that by that time the concentration of dioic acids was more or less constant.

[0049] Figure 2 shows the production of C_8-C_{14} unsaturated dicic acids individually and in total, using olive oil as the substrate. In this instance, the rate of production of dioic acids showed a less sudden increase but was continuing to rise at day 8.

[0050] In both cases, the larger dioic acids (C_{14}, C_{12}) constituted a greater percentage of the total than did the shorter chain dioic acids (C_{10}, C_8), although the precise product profile did vary between the two substrates (eg relatively more C_{10} product was obtained using sunflower oil as the substrate).

[0051] These data are also represented in tabular form in Table 1.

Example II - Use of pH to alter product profile

[0052] At a production pH of 7.4-7.6 the dominant species from oils (eg olive oil) containing C_{18} unsaturated fatty acids is the C_{14} unsaturated dioic acid.

[0053] However, if the production pH is decreased from 7.4-7.6 to around 7.1, the C_{12} unsaturated dioic acid becomes the dominant species. Fermentation was performed as detailed in the above examples until fermentation day 8 when the pH was dropped to 7.1 resulting in 'turn-over' at the C_{14} species and an increase in C_{12} production. The results are illustrated in Figure 3.

Table 1

	g/l Dioic Acid									
Fermentation Time (Days)	C14		C12		C10		C8		TOTAL C8-C14	
	SFO	CO	SFO	OO	SFO	OO	SFO	OO	SFO	OO
2	1.7	0.6	0.5	0.2	0.2	-	0.1	-	2.5	0.8
3	2.7	1.5	0.9	0.4	0.6	0.1	0.2	0.1	4.1	2.1
4	5.7	3.0	2.3	0.9	1.8	0.2	0.6	0.2	10.4	4.3
5	7.4	3.9	3.4	1.3	2.6	0.3	0.8	0.2	14.2	5.7
6	8.4	5.1	4.2	3.7	3.2	0.7	1.0	0.4	16.8	8.9
7	8.4	6.8	4.5	3.6	3.5	1.0	1.1	0.6	17.5	12
8	8.7	8.8	4.7	5.1	3.4	1.2	1.3	0.8	18.1	15.9

[0054] Figure 3 shows the production of C_8-C_{16} unsaturated dioic acids individually and in total, using olive oil as the substrate where the pH is adjusted on day 8 from 7.5 to 7.1. As noted in Figure 2, the total production of dioic acids continues to increase after day 8 when using olive oil as a substrate. However the product profile is significantly affected. Until day 8, the concentration of the C_{14} dioic acid continued to increase and was the most-concentrated dioic acid product. However, after that point, in the conditions of reduced pH, the concentration started to decline, whereas the C_{12} product continued to increase, such that after day 10 the C_{12} dioic acid represented the major product.

[0055] These data are also shown in tabular form in Table II.

[0056] This experiment shows that the product profile can be controlled to some extent by the production pH. The rate of C_8-C_{16} dioic production remains substantially linear after alteration of the production pH.

Example III - Tyrosinase Inhibition Assay

[0057] Inhibition of tyrosinase activity is used to identify potential skin whitening agents. Assays of tyrosinase inhibition were performed according to the methods of Humada and Mishima (Br. J. Derm. (1972) 86, 385-394).

[0058] All solutions were freshly prepared using 0.1 M sodium phosphate buffer (pH 6.8) as diluent. These were: 40mM Inhibitor stock solution: from which serial dilutions were made to obtain the following concentrations of 4.0, 0.4 and 0.04mM inhibitor,

Table II

Fermentation Day	Dioic Acid (g/l)					Total
	C_{16}	C_{14}	C_{12}	C_{10}	C_8	C_8-C_{16}
1	0.1	0.6	0.1	0	0	0.8
2	0.52	0.7	0.2	0	0	1.42
3	0.9	1.5	0.4	0.1	0.1	3.0
4	1.6	3.0	0.9	0.2	0.1	5.8
5	2.1	4.4	1.3	0.3	0.2	8.3

Table II (continued)

Fermentation Day	Dioic Acid (g/l)					Total
	C_{16}	C_{14}	C_{12}	C_{10}	C_8	C_8-C_{16}
6	1.5	5.5	2.7	0.7	0.4	10.8
7	0.6	7.1	3.6	1.0	0.6	12.9
8 Change of pH from 7.5-7.1	0.6	8.8	5.1	1.4	0.8	16.7
9	0.4	8.2	6.1	1.9	0.9	17.5
10	0.13	8.2	8.0	2.3	1.2	21.0
11	0.25	8.0	9.6	3.1	1.4	22.35
12	0.1	7.5	10.6	3.6	1.5	23.2
13	0.1	6.8	12.2	4.3	1.8	25.2

[0059] Salt solution: containing copper sulphate (100μM) and magnesium chloride (100mM),

[0060] Enzyme solution: 1ml mushroom tyrosinase (2000-4000 units per mg), and

[0061] Substrate solution: 48mg dihydioxyphenylalanine (DOPA)/100ml.

[0062] The enzyme and DOPA solutions were prepared immediately before use as they are light sensitive.

[0063] The inhibition of tyrosinase-catalysed oxidation of DOPA by dicarboxylic acids was followed spectrophotometrically by monitoring dopachrome formation at a wavelength of 492nm. The reaction was performed in 96-well microtitre plates with the addition of 30μl inhibitor (or buffer for the control), 50μl buffer and 20μl salt solution. DOPA (50μl) was added to start the reaction and each plate shaken for 30 seconds. Absorbance readings were taken after 10 minutes using a microtitre plate reader (Titertek Multiscan).

[0064] Results of the tyrosinase inhibition assay showed that, like azelaic acid, the unsaturated medium chain dioic acids were found to be effective tyrosinase inhibitors resulting in at least 50% inhibition of enzyme activity when present at 10mM concentration. This is surprising because azelaic acid is a saturated dioic acid and therefore has markedly different properties. Thus azelaic acid is a crystalline solid at room temperature whilst C_8/C_{10} unsaturated dioic acids are low melting-point oily substances.

[0065] It is possible that the enzyme thioredoxin reductase is a more significant enzyme than tyrosinase with respect to dioic acid-mediated inhibition of skin pigmentation. Recent research (described by Fitton & Goa in Drugs 41 (5), 780-798 (1991) has shown that azelaic acid inhibits thioredoxin reductase. In the light of the disclosure in this specification the skilled worker would therefore expect unsaturated dioic acids to be inhibitors of this enzyme as well.

Example IV - Inhibition of Melanin Production

[0066] In a further assay to complement Example III, the effects of unsaturated dioic acids on in vitro melanocyte cultures were investigated.

[0067] Pigment producing cells derived from a mammalian melanoma were grown in culture by standard methods. Preferred cell lines are B16 (disclosed in EP 0 338 104) or S-91 (e.g. ATCC COL 51.3. clone M-3) cells, but other lines or primary mouse or human melanocytes can be used.

[0068] Melanoma cells were grown in a complete cell culture medium (such as that described in EP 0 308 919) to approximately 1/3 confluence. The composition to be tested was then added to the culture medium.

[0069] The cells were cultured for a further period of 4 days and the amount of melanin produced was assayed by measuring the absorbance at 540 nm of the total melanin extracted from the culture medium and from the harvested cells.

[0070] The method described above was used to assess the ability of compositions comprising unsaturated dioic acids (C_{12}-C_{14} fraction, $C_{8\text{-}10}$ fraction, or mixed acids), at 0.1mM or 1.0mM, to reduce the amount of melanin produced by melanocyte cultures, relative to a negative control culture. Kojic acid (a substance used as a skin lightening agent) was used as a positive control. The results are shown in Figure 4, which is a bar chart showing the percentage reduction in melanin in the treated cultures compared to the untreated control.

[0071] It was found that the various dioic acid fractions had substantially similar properties in this respect.

Example V - Determination of antimicrobial activity

[0072] The Minimum Inhibitory Concentration (MIC) of each of various unsaturated dicarboxylic acid mixtures was

determined in the presence and absence of 10% Intralipid (Kabi Pharmacia, Inc.) using the agar dilution technique for susceptibility of 32 strains of Propionibacterium acnes and of 32 strains of various genera of aerobic bacteria. The method was as set out below.

[0073] A 5% stock solution for each agent was prepared by adding 10 grams of the dioic acid material to 200 milliliters of double strength Tryptic Soy Broth (TSB), (Baltimore Biological Laboratories). The pH of each solution was adjusted to 7.0 ± 0.2 with sodium hydroxide.

[0074] For each organic acid two sets of 200 ml capacity bottle/flasks were numbered 1 to 9. To each bottle was added 50 cc of double strength TSB. From the 5% stock solution, 50 cc of TSB were transferred to bottles #1 and #2. Serial transfers of 50 cc are made from bottle #2 through to boottle #8. Bottle #9 of each set contained only 50 cc of double strength TSB, without any dicarboxylic acid. To all 18 bottles were added 2 grams of granulated agar (BBL).

[0075] All bottles were autoclaved an 121°C, 15 psi for 15 minutes and then held at 50°C in a water bath.

[0076] To one set of bottles #1-9 were added 50 cc of hot, sterile water. The bottles were swirled to mix the contents and 25 cc was poured into each of four petri dishes and allowed to solidify. To the second set of bottles were added 50 cc of Intralipid (pre-warmed to 50°C). The contents were then mixed and poured as above.

[0077] Standard inocula of the test organisms were prepared by matching the bacterial suspension in 0,85% PSS (physiological saline solution) to a 0.5 McFarland Standard and diluting ten-fold to yield 10^7 CFU (colony forming units). The inocula were loaded into 32 wells of a steers replicator. The multi-prong inoculator delivers 0.001 to 0.002 cc resulting in a final inoculum of 10^4 CFU per spot.

[0078] Plates were inoculated from the lowest to highest concentration (to reduce the effects of "carry-over" of the inoculum), and allowed to dry. The plates were then inverted and incubated at 35°C for 24 hours. Plates inoculated with Propionibacterium acnes strains were incubate under anaerobic conditions for seven days at 35°C,

[0079] The agent-free control plates (#9) were examined at the end of the incubation for viability and signs of contamination. End-point MIC values were determined by observing the plate of lowest concentration of agent that inhibited visible micro-organism growth.

[0080] The results are summarised in Table III, which shows the MIC for medium chain unsaturated dioic acids ("Mixed dioic Acids", i.e. C_8-C_{14} mixed dioic acids), a C_{12} enriched fraction, and for the $C_{18:1}$ mono-unsaturated compound, compared with Azelaic acid, for a range of microorganisms. The data represent the results of experiments which were generally conducted on several different strains of each species (e.g. P. acnes strains ATCC 6919 and 29399 [ATCC stands for American Type Culture Collection]; Staph. aureus strains ATCC 25923, 35556 and 29213; Staph. epidermidis ATCC 35984, 31432 and 14490; Micrococcus sedentarius ATCC 27574; M. luteus ATCC 27141, 9341, and 15957; Brevibacterium epidermidis ATCC 35514; Corynebacterium minutissium ATCC 23347, 23348 and 23349).

[0081] The presence or absence of "Intralipid" had no significant effect. A slight difference was observed only for the $C_{18:1}$ mono-unsaturated compound, where there was a suggestion that intralipid increased the MIC for Staph. aureus and decreased the MIC for P. acnes and M. luteus.

TABLE 3

MIC AGAR DIFFUSION TEST FOR AZELAIC Vs UNSATURATED DIOIC ACIDS

Strain	Source	DICARBOXYLIC ACID TYPE (MIC %)					
		Azelaic acid	Mixed dioic acids ex Olive oil	C_{18} enriched mono unsat dioic acids ex Olive oil	Mixed dioic acids ex Sunflower oil	$C_{18:1}$ dioic acid ex Oleic acid	$C_{18:1}$ dioic acid ex Oleic acid + "Intralipid"
Propionibacterium acnes	ATCC 6919	1.25	0.31	0.31	0.31	0.01	0.02
Propionibacterium acnes	ATCC 29399	1.25	0.31	0.31	0.62	0.01	0.02
Staphylococcus aureus	ATCC 25923	2.5	0.62	1.25	1.25	0.07	0.15
Staphylococcus aureus	ATCC 35556	2.5	0.31	0.31	0.62	0.07	0.31
Staphylococcus aureus	ATCC 29213	2.5	1.25	0.62	2.5	0.15	0.31
Staphylococcus epidermidis	ATCC 35984	>2.5	1.25	0.62	2.5	0.31	0.31
Staphylococcus epidermidis	ATCC 31432	>2.5	1.25	0.62	2.5	0.31	0.31
Staphylococcus epidermidis	ATCC 14990	>2.5	1.25	0.62	2.5	0.31	0.31
Micrococcus sedentarius	ATCC 27574	2.5	0.15	0.62	0.62	0.15	0.07
Micrococcus luteus	ATCC 27141	2.5	0.15	0.62	0.62	0.15	0.07
Micrococcus luteus	ATCC 9341	>2.5	1.25	0.62	0.62	0.15	0.07
Micrococcus luteus	ATCC 15957	>2.5	1.25	0.62	0.62	0.15	0.07
Brevibacterium epidermidis	ATCC 35514	>2.5	0.62	0.62	0.62	0.15	0.15
Brevibacterium epidermidis	NCDO 2285	>2.5	0.62	0.62	0.62	0.15	0.15
Corynebacterium minutissium	ATCC 23347	>2.5	0.62	0.62	0.62	0.15	0.15
Corynebacterium minutissium	ATCC 23348	>2.5	0.62	0.62	0.62	0.15	0.15
Corynebacterium minutissium	ATCC 23349	>2.5	0.62	0.62	0.62	0.15	0.15
Pseudomonas aeruginosa	ATCC 27853	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5
Escherichia coli	ATCC 25922	2.5	>2.5	>2.5	>2.5	>2.5	>2.5
Candida albicans	ATCC 18804	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5

P. acnes is the main causative agent of acne, thus efficacy against this organism indicates a usefulness in the prevention and treatment of acne. Staphylococcus aureus is a pathogenic Gram positive organism commonly associated with boils and abscesses. Candida albicans is a resistant yeast included as a negative control. Pseudomonas aeruqinosa and Escherichia coli are both common Gram negative organisms.

[0082] Unexpectedly, in all cases the unsaturated dioic acids (both medium chain and C18 compounds) were more active than azelaic acid. The compounds were particularly effective against P. acnes, Staph. aureus, and Staph. epi-

dermidis. In particular the C_{18} compound exhibit d anti-microbial activity many times greater than that for azelaic acid.

[0083] A similar experiment was performed, using the same method, to compare the degree of inhibitory activity (for P. acnes) of azelaic acid, the $C_{18:1}$ dioic acid (obtained from a substrate comprising oleic acid), a mixture of $C_{18:1}$ fatty (mono-carboxylic) acid, the corresponding $C_{18:1}$ and $C_{18:2}$ dioic acids (obtained from a substrate comprising Linoleic acid), and the $C_{16:1}$ dioic acid (obtained from a substrate comprising palmitoleic acid). The results are shown below in Table IV. The MICs for azelaic acid and the $C_{18.1}$ dioic acid were essentially as before, confirming the previous results. The corresponding fatty acid had very little activity. Thus, the $C_{18.1}$ dioic acid then has almost 100x the activity of azelaic acid, although the equivalent fatty acid (oleic acid) is less active than azelaic acid.

[0084] In experiment 4, the degree of inhibition of the azelaic acid control, and therefore of the test samples also, was slightly less than that observed in previous experiments

TABLE 4

MIC's FOR LONG CHAIN DIOIC ACIDS AGAINST P.ACNES ATCC 25746 Vs AZELAIC ACID AND OLEIC ACID CONTROLS				
Test Material	MIC (%)			
	Expt. 1	Expt. 2	Expt. 3	Expt. 4
Azelaic acid (control)	5	5	5	>5
$C_{18.1}$ Fatty acid (control)	nd	nd	>5	nd
$C_{18.1}$ dioic acid ex Oleic acid	0.04	0.04	0.04	0.08
$C_{18.2/18.1}$ dioic acid ex 60% Linoleic acid	nd	nd	nd	0.08
$C_{16.1}$ dioic acid ex Palmitoleic acid	nd	nd	nd	0.16

[0085] In experiment 4 the degree of inhibition of the azelaic acid control, and therefore of the test samples also, was slightly less than previously observed. The most probable reason for this was the increased incubation period of 18 days as against 7 days for the earlier work.

Claims

1. A pharmaceutical or cosmetic composition comprising an unsaturated aliphatic dioic acid, and/or a derivative of an unsaturated aliphatic dioic acid wherein the unsaturated aliphatic dioic acid contains from 8 to 22 carbon atoms (inclusive) and/or the derivative is a derivative of an unsaturated aliphatic dioic acid containing from 15 to 22 carbon atoms (inclusive) in the main hydrocarbon chain; other than a composition in which the sole active unsaturated aliphatic dioic acid is trans-2-dodecenedioic acid.

2. A composition according to claim 1, comprising one or more unsaturated C_{16} or C_{18} dioic acids and/or a derivative thereof.

3. A composition according to claim 1 or 2, having antimierobial activity.

4. A composition according to claim 1, 2 or 3, being active against *Propionibacterium acnes* and *Staphylococcus aureus*.

5. A composition according to any one of claims 1 to 4, having skin-lightening activity.

6. A composition according to any one of claims 1 to 5, wherein the dioic acid derivative is an alcohol, a substituted or unsubstituted amide, a salt or a mono- or diester.

7. A composition according to any one of claims 1 to 6, suitable for topical application to the skin.

8. A composition according to any one of claims 1 to 7. wherein the composition comprises from 0.001% to 20% by weight of unsaturated dioic acid and/or derivative thereof.

9. A composition according to any one of claims 1 to 8, wherein the composition comprises from 0.01% to 1% by

weight of unsaturated dioic acid and/or derivative thereof.

10. A method of treating human skin for cosmetic purposes, comprising use of an effective amount of a composition in accordance with any one of claims 1 to 9.

11. A cosmetic method of treating human skin in accordance with claim 10, for the purpose of treating a condition caused, maintained or exacerbated by *Propionibacterium acnes* and/or *Staphylococcus aureus*.

12. A cosmetic method of treating human skin in accordance with claim 10 or 11, for the treatment of one or more of the following: acne; wrinkles; dermatosis; hyper-pigmentary dermatosis; eczema; rosacea; lentigo; seborrhoea; impetigo; dandruff; and body malodour.

13. A method of lightening skin, comprising the use of an unsaturated aliphatic dioic acid or a derivative of an unsaturated dioic acid, wherein the unsaturated aliphatic dioic acid contains from 8 to 22 carbon atoms (inclusive) or wherein the derivative is a derivative of an unsaturated aliphatic dioic acid containing from 15 to 22 carbon atoms (inclusive) in the main hydrocarbon chain

14. Use of an unsaturated aliphatic dioic acid or a derivative of unsaturated aliphatic dioic acid as an active cosmetic substance, wherein the unsaturated aliphatic dioic acid contains from 8 to 22 carbon atoms (inclusive) or wherein the derivative is a derivative of an unsaturated aliphatic dioic acid containing from 15 to 22 carbon atoms (inclusive) in the main hydrocarbon chain.

15. An unsaturated aliphatic dioic acid or a derivative of unsaturated aliphatic dioic acid, wherein the unsaturated aliphatic dioic acid contains from 8 to 22 carbon atoms (inclusive) or wherein the derivative is a derivative of an unsaturated aliphatic dioic acid containing from 15 to 22 carbon atoms (inclusive) in the main hydrocarbon chain and wherein the unsaturated aliphatic dioic acid is other than trans-2-dodecenedioic acid, for use as an active therapeutic substance.

16. A substance according to claim 15, comprising a C_{16} or C_{18} unsaturated aliphatic dioic acid or a derivative thereof.

17. Use of an unsaturated aliphatic dioic acid or derivative of an unsaturated aliphatic dioic acid in the manufacture of a medicament for use as an active anti-microbial agent.

18. Use of an unsaturated aliphatic dioic acid or derivative of an unsaturated aliphatic dioic acid as an active skin lightening agent.

19. Use of a C_{16} or C_{18} unsaturated aliphatic dioic acid or a derivative thereof according to claim 14, 17 or 18.

20. A method of making a therapeutic or cosmetic composition for topical application to the skin, comprising mixing an effective amount of an unsaturated aliphatic dioic acid or a derivative thereof with a dermatologically acceptable therapeutic or cosmetic carrier; wherein the unsaturated aliphatic dioic acid contains from 8 to 22 carbon atoms (inclusive) or wherein the derivative is a derivative of an unsaturated aliphatic dioic acid containing from 15 to 22 carbon atoms (inclusive) in the main hydrocarbon chain; and wherein the unsaturated aliphatic dioic acid is other than trans-2-dodecenedioic acid.

21. A method according to claim 20, wherein the unsaturated aliphatic dioic acid or derivative thereof is a C_{16} or C_{18} unsaturated dioic acid or derivative.

22. A method of preparing an unsaturated aliphatic dioic acid, comprising limited chain-shortening β oxidation of an unsaturated aliphatic substrate using a yeast propagated in a growth medium, wherein the yeast comprises *Candida cloacae* strain 5GLA12.

23. A method according to claim 22, wherein the substrate comprises a C_{16}-C_{22} unsaturated compound.

24. A method according to claim 22 or 23, wherein the substrate comprises oleic, linoleic, linolenic or arachidonic acids.

25. A method according to any one of claims 22 to 24, wherein the substrate comprises a triglyceride.

26. A method according to any one of claims 22 to 25, wherein the substrate comprises olive oil, sunflower oil or castor oil.

27. A method according to any one of claims 22 to 26, wherein the yeast is not grown under conditions of nitrogen-limitation.

28. A method according to any one of claims 22 to 27, wherein alteration of pH affects dioic acid·product profile of the process.

Patentansprüche

1. Pharmazeutische oder kosmetische Zusammensetzung, die eine ungesättigte aliphatische Dicarbonsäure und/ oder ein Derivat einer ungesättigten aliphatischen Dicarbonsäure umfaßt, wobei die ungesättigte aliphatische Dicarbonsäure von 8 bis einschließlich 22 Kohlenstoffatome enthält und/ oder das Derivat ein Derivat einer ungesättigten aliphatischen Dicarbonsäure ist, die von 15 bis einschließlich 22 Kohlenstoffatome in der Kohlenwasserstoffhauptkette enthält, wobei die Zusammensetzung keine solche ist, in der die einzige aktive ungesättigte aliphatische Dicarbonsäure trans-2-Dodecendicarbonsäure ist.

2. Zusammensetzung nach Anspruch 1, die eine oder mehrere ungesättigte C_{16}- oder C_{18}-Dicarbonsäuren und/oder ein Derivat davon enthält.

3. Zusammensetzung nach Anspruch 1 oder 2, die antimikrobielle Wirksamkeit aufweist.

4. Zusammensetzung nach Anspruch 1, 2 oder 3, die gegen *Propionibacterium acnes* und *Staphylococcus aureus* aktiv ist.

5. Zusammensetzung nach irgendeinem der Ansprüche 1 bis 4, die hautbleichende Wirksamkeit aufweist.

6. Zusammensetzung nach irgendeinem der Ansprüche 1 bis 5, bei der das Dicarbonsäurederivat ein Alkohol, ein substituiertes oder unsubstituiertes Amid, ein Salz oder ein Mono- oder Diester ist.

7. Zusammensetzung nach irgendeinem der Ansprüche 1 bis 6, die zum topischen Anwenden auf der Haut geeignet ist.

8. Zusammensetzung nach einem der Ansprüche 1 bis 7, worin die Zusammensetzung von 0,001 bis 20 Gew.-% ungesättigte Dicarbonsäure und/oder Derivat davon umfaßt.

9. Zusammensetzung nach einem der Ansprüche 1 bis 8, worin die Zusammensetzung von 0,01 bis 1 Gew.-% ungesättigte Dicarbonsäure und/oder Derivat davon umfaßt.

10. Verfahren zur Behandlung der menschlichen Haut für kosmetische Zwecke, das die Verwendung einer wirksamen Menge einer Zusammensetzung nach irgendeinem der Ansprüche 1 bis 9 umfaßt.

11. Kosmetisches Verfahren zur Behandlung der menschlichen Haut nach Anspruch 10 zum Zwecke der Behandlung eines Zustands, der von *Propionibacterium acnes* und *Staphylococcus aureus* bewirkt, aufrechterhalten oder verschlimmert wird.

12. Kosmetisches Verfahren zur Behandlung der menschlichen Haut nach Anspruch 10 oder 11, zur Behandlung von einem oder mehreren von den folgenden: Akne, Falten, Dermatose, hyper-pigmentäre Dermatose, Ekzem, Rosacea, Lentigo, Seborrhoea, Impetigo, Dandruff und unangenehmem Körpergeruch.

13. Verfahren zur Bleichung der Haut, das die Verwendung einer ungesättigten aliphatischen Dicarbonsäure oder eines Derivats einer ungesättigten Dicarbonsäure umfaßt, wobei die ungesättigte aliphatische Dicarbonsäure von 8 bis einschließlich 22 Kohlenstoffatome enthält oder worin das Derivat ein Derivat einer ungesättigten aliphatischen Dicarbonsäure ist, die von 15 bis einschließlich 22 Kohlenstoffatome in der Kohlenwasserstoffhauptkette enthält.

14. Verwendung einer ungesättigten aliphatischen Dicarbonsäure oder eines Derivats einer ungesättigten aliphatischen Carbonsäure als kosmetische Wirksubstanz, wobei die ungesättigte aliphatische Dicarbonsäure von 8 bis einschließlich 22 Kohlenstoffatome enthält, oder worin das Derivat ein Derivat einer ungesättigten aliphatischen Dicarbonsäure ist, die von 15 bis einschließlich 22 Kohlenstoffatome in der Kohlenwasserstoffhauptkette enthält.

15. Eine ungesättigte aliphatische Dicarbonsäure oder ein Derivat einer ungesättigten aliphatischen Dicarbonsäure, wobei die ungesättigte aliphatische Dicarbonsäure von 8 bis einschließlich 22 Kohlenstoffatome enthält oder worin das Derivat ein Derivat einer ungesättigten aliphatischen Dicarbonsäure ist, die von 15 bis einschließlich 22 Kohlenstoffatome in der Kohlenwasserstoffhauptkette enthält, und wobei die ungesättigte aliphatische Dicarbonsäure nicht trans-2-Dodecendicarbonsäure ist, zur Verwendung als aktive therapeutische Substanz.

16. Substanz nach Anspruch 15, die eine C_{16}- oder C_{18}- ungesättigte aliphatische Dicarbonsäure oder ein Derivat davon umfaßt.

17. Verwendung einer ungesättigten aliphatischen Dicarbonsäure oder eines Derivats einer ungesättigten aliphatischen Dicarbonsäure bei der Herstellung eines Medikaments zur Verwendung als aktives antimikrobielles Mittel.

18. Verwendung einer ungesättigten aliphatischen Carbonsäure oder eines Derivats einer ungesättigten aliphatischen Dicarbonsäure als Wirkstoff zur Hautbleichung.

19. Verwendung einer C_{16}- oder C_{18}- ungesättigten aliphatischen Dicarbonsäure oder eines Derivats davon nach Anspruch 14, 17 oder 18.

20. Verfahren zur Herstellung einer therapeutischen oder kosmetischen Zusammensetzung für die topische Anwendung auf der Haut, das das Mischen einer wirksamen Menge einer ungesättigten aliphatischen Dicarbonsäure oder eines Derivats davon mit einem dermatologisch annehmbaren therapeutischen oder kosmetischen Träger umfaßt, wobei die ungesättigte aliphatische Dicarbonsäure von 8 bis einschließlich 22 Kohlenstoffatome enthält oder worin das Derivat ein Derivat einer ungesättigten aliphatischen Dicarbonsäure ist, die von 15 bis einschließlich 22 Kohlenstoffatome in der Kohlenwasserstoffhauptkette enthält, und wobei die ungesättigte aliphatische Dicarbonsäure nicht trans-2-Dodecendicarbonsäure ist.

21. Verfahren nach Anspruch 20, bei dem die ungesättigte aliphatische Dicarbonsäure oder das Derivat davon eine C_{16}- oder C_{18}- ungesättigte Dicarbonsäure oder ein Derivat davon ist.

22. Verfahren zur Herstellung einer ungesättigten aliphatischen Dicarbonsäure, das eine limitierte kettenverkürzende β-Oxidation eines ungesättigten aliphatischen Substrats unter Verwendung einer Hefe umfaßt, die in einem Nährmedium vermehrt wird, wobei die Hefe *Candida cloacae* 5GLA12 umfaßt.

23. Verfahren nach Anspruch 22, bei dem das Substrat eine C_{16}-C_{22}- ungesättigte Verbindung umfaßt.

24. Verfahren nach Anspruch 22 oder 23, wobei das Substrat Öl-, Linol-, Linolen- oder Arachidonsäuren umfaßt.

25. Verfahren nach irgendeinem der Ansprüche 22 bis 24, bei dem das Substrat ein Triglycerid umfaßt.

26. Verfahren nach irgendeinem der Ansprüche 22 bis 25, bei dem das Substrat Olivenöl, Sonnenblumenöl oder Rizinusöl umfaßt.

27. Verfahren nach irgendeinem der Ansprüche 22 bis 26, wobei die Hefe nicht unter stickstofflimitierenden Bedingungen gezüchtet wird.

28. Verfahren nach irgendeinem der Ansprüche 22 bis 27, wobei die Änderung des pH das Dicarbonsäure-Produktprofil des Verfahrens beeinflußt.

Revendications

1. Composition pharmaceutique ou cosmétique comprenant un acide aliphatique dioïque insaturé, et/ou un dérivé d'un acide aliphatique dioïque insaturé, où l'acide aliphatique dioïque insaturé contient de 8 à 22 atomes de car-

bone, limites comprises, et/ou le dérivé est un dérivé d'un acide aliphatique dioïque insaturé contenant de 15 à 22 atomes de carbone, limites comprises, dans sa chaîne principale ; autre qu'une composition dans laquelle l'acide aliphatique dioïque insaturé actif unique est l'acide trans-2-dodécènedioïque.

2. Composition selon la revendication 1, qui comprend un ou plusieurs acides dioïques en C_{16} ou en C_{18} et/ou un dérivé de ceux-ci.

3. Composition selon la revendication 1 ou 2, qui possède une activité antimicrobienne.

4. Composition selon la revendication 1, 2 ou 3, qui est active contre ***Propionibacterium acnes*** et ***Staphylococcus aureus.***

5. Composition selon l'une quelconque des revendications 1 à 4, qui possède une activité d'éclaircissement de la peau.

6. Composition selon l'une quelconque des revendications 1 à 5, dans laquelle le dérivé d'acide dioïque est un alcool, un amide substitué ou non-substitué, un sel, ou un mono- ou un diester.

7. Composition selon l'une quelconque des revendications 1 à 6, qui convient à une application topique sur la peau.

8. Composition selon l'une quelconque des revendications 1 à 7, dans laquelle la composiion comprend de 0,001 à 20 % en poids d'un acide dioïque insaturé et/ou d'un dérivé de celui-ci.

9. Composition selon l'une quelconque des revendications 1 à 8, dans laquelle la composition comprend de 0,01 à 1 % en poids d'un acide dioïque insaturé et/ou d'un dérivé de celui-ci.

10. Procédé pour traiter la peau humaine à des fins cosmétiques, qui comprend l'utilisation d'une quantité efficace d'une composition selon l'une quelconque des revendications 1 à 9.

11. Procédé cosmétique pour traiter la peau humaine selon la revendication 10, aux fins de traiter une affection provoquée, entretenue ou exacerbée par ***Propionibacterium acnes*** et/ou ***Staphylococcus aureus.***

12. Procédé cosmétique pour traiter la peau humaine selon les revendication 10 ou 11, pour le traitement d'une ou plusieurs des affections suivantes : acné, rides, dermatose, dermatose hyperpigmentaire, eczéma, acné rosacée, lentigo, séborrhée, impétigo, pellicules et mauvaises odeurs corporelles.

13. Procédé d'éclaircissement de la peau, qui comprend l'utilisation d'un acide aliphatique dioïque insaturé ou d'un dérivé d'un acide dioïque insaturé, où l'acide aliphatique dioïque insaturé contient de 8 à 22 atomes de carbone, limites comprises, ou encore où le dérivé est un dérivé d'un acide aliphatique dioïque insaturé contenant de 15 à 22 atomes de carbone, limites comprises, dans sa chaîne principale.

14. Utilisation d'un acide aliphatique dioïque insaturé ou d'un dérivé d'un acide aliphatique dioïque insaturé en tant que substance active cosmétique, où l'acide aliphatique dioïque insaturé contient de 8 à 22 atomes de carbone, limites comprises, ou encore où le dérivé est un dérivé d'un acide aliphatique dioïque insaturé contenant de 15 à 22 atomes de carbone, limites comprises, dans sa chaîne principale.

15. Acide aliphatique dioïque insaturé ou dérivé d'un acide aliphatique dioïque insaturé, où l'acide aliphatique dioïque insaturé contient de 8 à 22 atomes de carbone, limites comprises, ou encore où le dérivé est un dérivé d'un acide aliphatique dioïque insaturé contenant de 15 à 22 atomes de carbone, limites comprises, dans sa chaîne principale, et où l'acide aliphatique dioïque insaturé est autre que l'acide trans-2-dodécènedioïque, pour utilisation en tant que substance active thérapeutique.

16. Substance selon la revendication 15, qui comprend un acide aliphatique dioïque insaturé en C_{16} ou C_{18}, ou un dérivé de celui-ci.

17. Utilisation d'un acide aliphatique dioïque insaturé ou d'un dérivé d'un acide aliphatique dioïque insaturé dans la préparation d'un médicament pour utilisation en tant qu'agent actif antimicrobien.

18. Utilisation d'un acide aliphatique dioïque insaturé ou d'un dérivé d'un acide aliphatique dioïque insaturé en tant qu'agent actif d'éclaircissement de la peau.

19. Utilisation d'un acide aliphatique dioïque insaturé en C_{16} ou C_{18} ou d'un dérivé de celui-ci selon l'une quelconque des revendications 14, 17 ou 18.

20. Procédé de préparation d'une composition thérapeutique ou cosmétique pour application topique sur la peau, qui consiste à mélanger une quantité eficace d'un acide aliphatique dioïque insaturé ou d'un dérivé de celui-ci à un excipient cosmétique ou pharmaceutique acceptable d'un point de vue dermatologique, où l'acide aliphatique dioïque insaturé contient de 8 à 22 atomes de carbone, limites comprises, ou encore où le dérivé est un dérivé d'un acide aliphatique dioïque insaturé contenant de 15 à 22 atomes de carbone, limites comprises, dans sa chaîne principale ; et où l'acide aliphatique dioïque insaturé est autre que l'acide trans-2-dodécènedioïque.

21. Procédé selon la revendication 20, dans lequel l'acide aliphatique dioïque insaturé ou le dérivé de celui-ci est un acide dioïque insaturé en C_{16} ou C_{18} ou un dérivé de celui-ci.

22. Procédé de préparation d'un acide aliphatique dioïque insaturé, qui comprend une β-oxydation limitée, avec raccourcissement de chaîne, d'un substrat aliphatique insaturé, par utilisation d'une levure que l'on a fait proliférer dans un milieu de croissance, où la levure comprend la souche 5GLA12 de ***Candida cloacae***.

23. Procédé selon la revendication 22, dans lequel le substrat comprend un composé insaturé en C_{16}-C_{22}.

24. Procédé selon la revendication 22 ou 23, dans lequel le substrat comprend les acides oléique, linoléique, linolénique ou arachidonique.

25. Procédé selon l'une quelconque des revendications 22 à 24, dans lequel le substrat comprend un triglycéride.

26. Procédé selon l'une quelconque des revendications 22 à 25, dans lequel le substrat comprend de l'huile d'olive, de l'huile de tournesol ou de l'huile de ricin.

27. Procédé selon l'une quelconque des revendications 22 à 26, dans lequel la levure ne prolifère pas dans des conditions de limitation d'azote.

28. Procédé selon l'une quelconque des revendications 22 à 27, dans lequel une modification du pH affecte le profil des acides dioïques produits par le procédé.

FIGURE 1

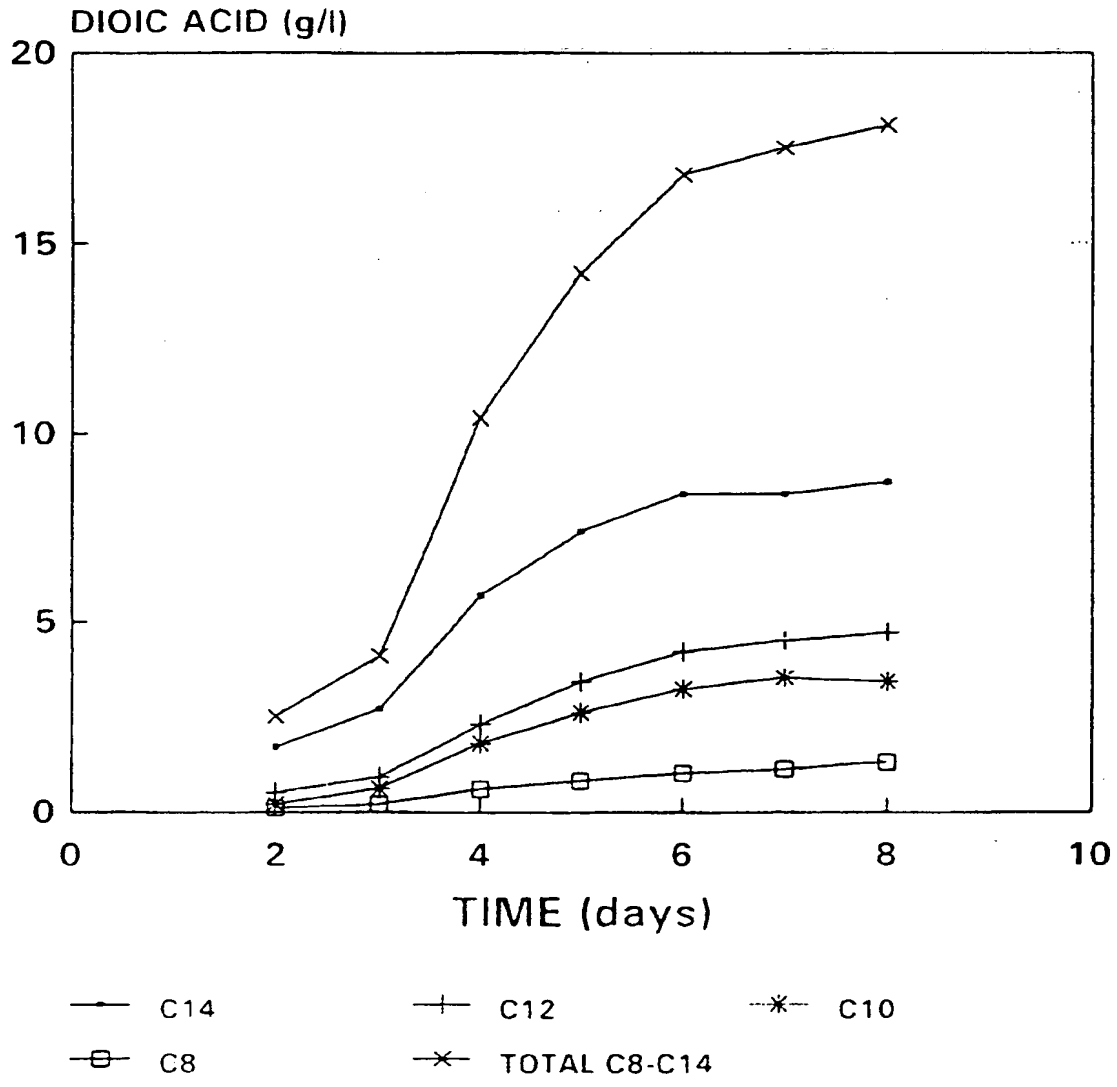

DIOIC ACID (g/l)
20
15
10
5
0
0
2
4
6
8
10
TIME (days)
C14
C12
C10
C8
TOTAL C8-C14

FIGURE 2

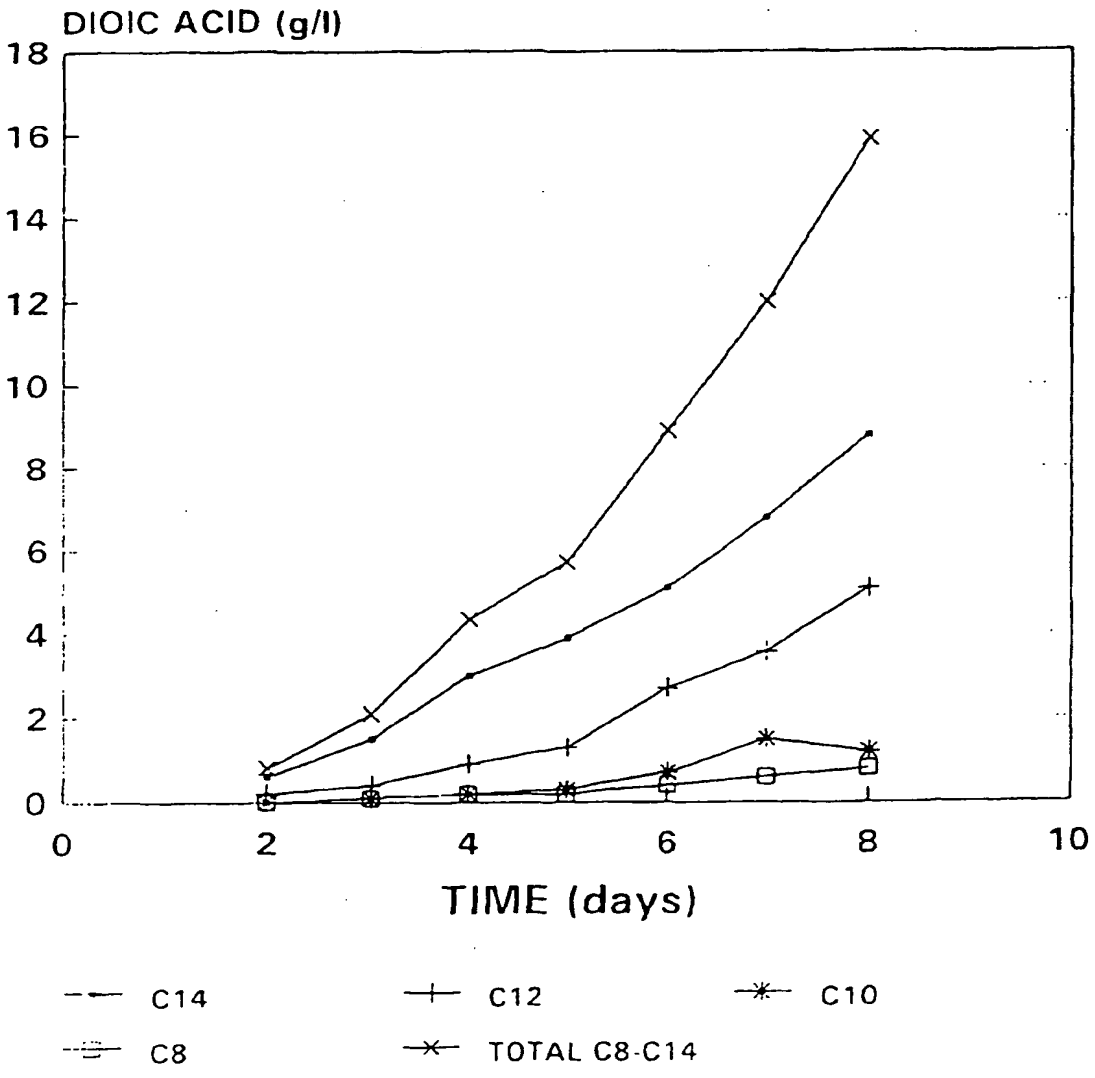
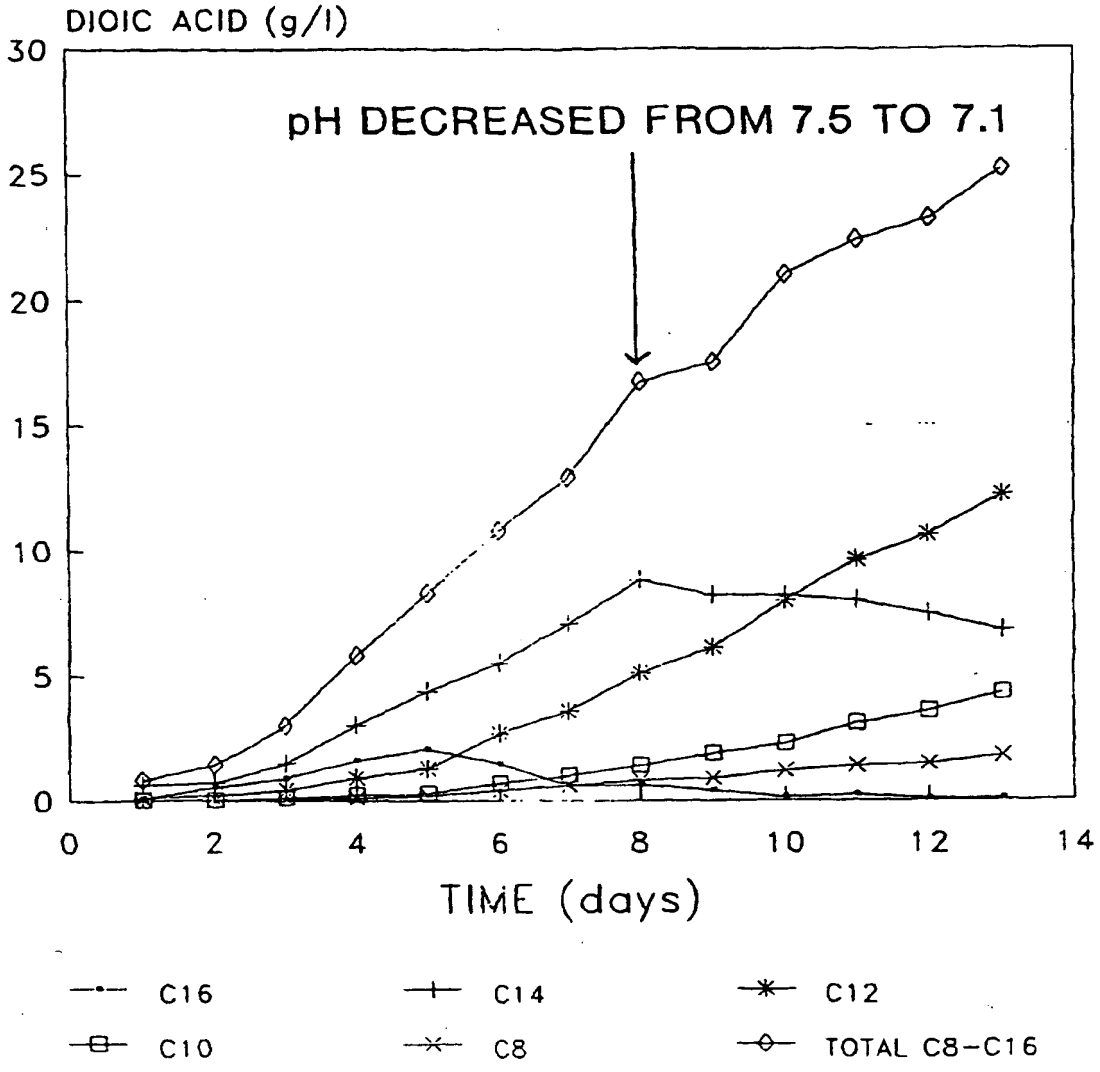

DIOIC ACID (g/l)
18
16
14
12
10
8
6
4
2
0
0
2
4
6
8
10
TIME (days)
C14
C12
C10
C8
TOTAL C8-C14

FIGURE 3


DIOIC ACID (g/l)
30
25
20
15
10
5
0
pH DECREASED FROM 7.5 TO 7.1
0
2
4
6
8
10
12
14
TIME (days)
C16
C14
C12
C10
C8
TOTAL C8-C16

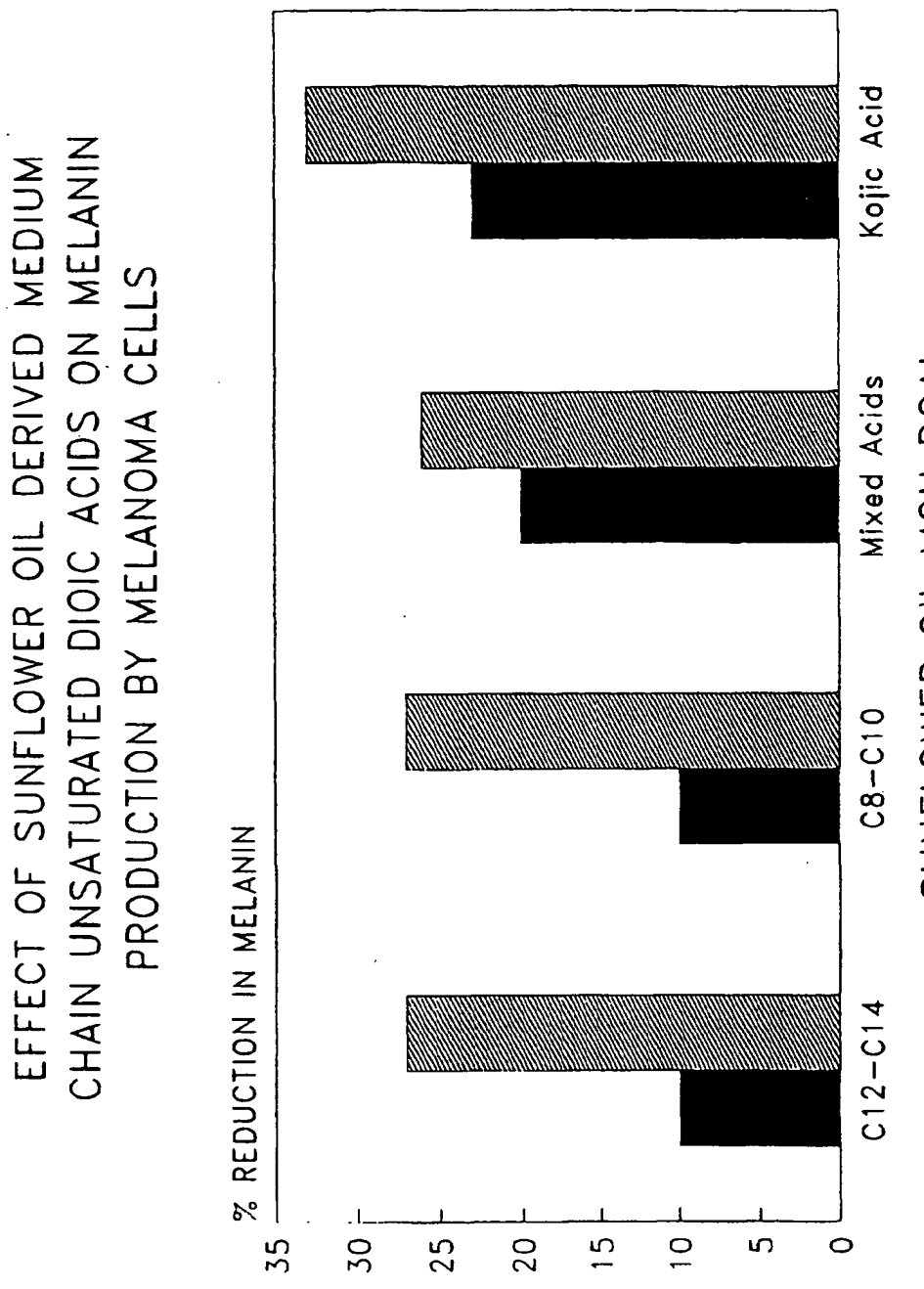


Figure 4: Kojic acid is the control.